MORPHOLOGICAL AND CHEMICAL VARIABILITY OF LEBANESE CAROB VARIETIES

Amira Haddarah
Université de Lorraine, LIBio (Laboratoire d'Ingénierie des biomolécules),Vandœuvre-lès-Nancy, France
Université Libanaise, Faculté d'Agronomie, Dekwaneh, Liban
Université Libanaise, Faculté des Sciences, Laboratoire MCEMA, EDST, Hadath, Liban
Ali Ismail
Ali Bassal
Tayssir Hamieh
Université Libanaise, Faculté d'Agronomie, Dekwaneh, Liban
Université Libanaise, Faculté des Sciences, Laboratoire MCEMA, EDST, Hadath, Liban
Irina Ioannou
Mohamed Ghoul
Université de Lorraine, LIBio (Laboratoire d'Ingénierie des biomolécules),Vandœuvre-lès-Nancy, France

Abstract

Morphological variation (pod length, width, thickness, seed number, volume, weight and specific gravity measurements) and chemical composition (protein, sugar, fiber, ash and total phenol content) of nine carob varieties from different regions of Lebanon were investigated. The obtained results showed that these two criteria exhibit significant differences ($p<0.05$) allowing thus to establish correlations between morphological aspects and location, mainly latitude. Moreover, the principal component analysis (PCA) of the data allowed separating these varieties in two grouped and two ungrouped populations.

Keywords: Carob, Morphological characteristics, *Ceratonia siliqua*, Polyphenol, Sugar

Abbreviations: A1: Akkari 1, A2: Akkari 2, Ah: Ahmar, Bal: Baladi, Bar: Barri, H_2SO_4: sulfuric acid, J1: Jnoubi1, J2: Jnoubi2, Kh: khachabi, Na_2CO_3:

sodium carbonate, PCA: principal component analysis, p: probability, SM: Sandali Makdissi.

Introduction

Carob tree is a long-lived evergreen tree (*Ceratonia siliqua L.*) that has been widely cultivated around the world over the years and described as a plant with a large adaptability in the Mediterranean area (Lossaint, 1973; Batlle and Tous, 1997). It is found spontaneously on the coastal areas up to 800m on the western slopes of Lebanon Mount.

Carob tree has an economic and environmental importance in Lebanon. It is used in reforestation of arid and degraded areas and also as for ornamental purposes (Winer, 1980; Girolamo and Laura, 2002). The pulp and the seeds have some interesting properties and are often used in food and pharmacological industry (Batlle, 1997; Markis et Kefalas, 2004). The pulp content in the pod ranges from 73 to 95% (Caja et al. 1988; Marakis et al. 1988; Shawakfeh et Ereifej, 2005). Carob pods are also characterized by high sugar content (500 g/kg) (NAS, 1979; Petit and Pinilla, 1995; Batlle and Tous, 1997; Markis and Kefalas, 2004). Moreover, carob pods contain appreciable amount of fiber (4.2-39.8%), depending on the type of the extracted fiber (Thomson, 1971; Shawakfeh et Ereifej, 2005). Carob pulp is a good source of polyphenols (mainly tannins 16-20%) (Batlle and Tous, 1997; Owen et al., 2003; Biner et al., 2007), and protein (2.7-7.6%) but it is poor in lipid (0.4-0.8%).

The pulp and the seeds are valorized in different applications. Hariri et al. (2009) reported that the pod fiber content play a role in hypocholesterolemic and hypoglycemic regulation, whereas phenolic compounds can be used as antioxidant additive. Moreover, the locust bean gum (additive E 410) extracted from the endosperm of seeds is used as stabilizer and thickening agents in food industry (Batlle and Tous, 1997; Neukom, 1989). Moreover, seeds powder can be used in baby foods to prevent vomiting (FAO, 2001). The locust bean gum is also applied in pharmaceutical industry as drug delivery (Sandolo et al., 2007).

Chemical composition of carob had been studied extensively for different countries of the Mediterranean area. It had been observed that this composition is depending not only on technological factors such as the extraction and analytical methodologies, but also on the genotype of the plant, the geographical origin, the climate conditions and the harvesting and storage procedures (Batlle & Tous, 1997; Biner et al., 2007; Owen et al., 2003; Naghmouchi et al, 2009; Sidina et al., 2009). However, in spite of the great interest to carob and their use in different applications, few studies are available on Lebanese carob. So to select the best varieties, an intensive investigation on the morphological and chemical composition for the

different carob Lebanese varieties is needed. These studies will allow understanding correlations between fruit, seed characteristics and geographical coordinates in order to propose the best characteristics cultivars that can be helpful for the development of new orchards with the best agro industrial profitability. Based on the above considerations, the aim of the current study was to assess morphological characteristics and chemical composition of wild and cultivated types of carob in Lebanon and to establish correlation between morphological parameters and environmental factors such as altitude, latitude and longitude.

Materials and methods

Sampling and experimental method for morphological-and chemical analysis

Nine Lebanese carob populations, including a wild type, were randomly collected from various geographic sites with different altitudes of the Lebanese territories (Fig. 1, Table 1). Ten pods from each variety of the carob were randomly chosen to measure the different parameters. Populations were located on an axis North-south on different geographical regions (Fig. 1). J1, J2, Bar and SM come from south Lebanon with different geographical heights. Khachabi is in the middle of the country near the Mount Lebanon. Ahmar, Baladi, Akkari are located only in the north of Lebanon. Moreover, geographical repartitions of carob were accompanied with GPS locator in order to see whether there is any correlation between chemical and location parameters (Table 1).

Morphological parameters

For ten pods of each variety, following parameters have been measured: weight, length, width, thickness, volume, number of seeds, specific gravity, size index, number of seeds / pod, seeds weight / pod and % seed / pod.

Length (cm) of pod was measured using a measuring tape, whereas width (cm) was assessed with the Vernier caliper (top, middle, and bottom of pod). Thickness (cm) was evaluated with Iwanson gauge (1/10 mm) where three parts of the top, middle and bottom of the pod were covered. Weight (g) of pods and kernels were taken using a top-loading balance. Volume (cm^3) was estimated by submerging carob pod in a known volume of water inside a graduated cylinder (1000 cm^3). Size index was determined as the ratio of length over width and specific gravity is the density (pod Mass / pod volume) of carob pod over density of water.

Chemical analysis

To determine the chemical composition of carob pulp (total polyphenols, total sugars, fiber, protein and ash content), samples from seedless pods of morphological measurements were crashed, and then grounded into powder using a hammer mill (diameter less than 0.5 mm).

Extracts were prepared as follows: 1 g of carob powder was mixed with 20 ml of water and 20 ml of acetone in a reactor at room temperature (20 - 22 ° C, 30 min).

Determination of total phenols

Total phenolic compounds were determined colorimetrically at 660nm and expressed as gallic acid equivalents, according to the method described by Singleton et al. (1965). Samples were added to Folin–Cioccalteu reagent and $CaCO_3$ solution and placed in the dark for 15 min before spectrophotometric analysis.

Determination of sugar content

Total sugars were determined colorimetrically at 480 nm according to the method described by DUBOIS et al. (1956). Standards were prepared with glucose solutions at different concentrations.

Determination of protein content

Total nitrogen of carob powder was determined according to the AOAC official method 955.04 (AOAC, 2007) using a MacroKjeldahl digestion and distillation apparatus.

Determination of fiber content

Four grams of carob powder were digested with 200 ml of 5% HCL for 30 minutes. The mixture was filtered and washed with hot water. Then, residue was digested with 200 ml of 5% NaOH under reflux for 30 min. The mixture was filtered and washed with distilled water until neutrality of pH. The material was washed with 20 ml of ethyl alcohol and 20 ml of ethyl ether. Finally, the residue was dried at 100 ˚ C for two hours and the residual mass was considered fibers (De Pádua et al, 2004).

Determination of ash and moisture contents

The ash content of the carob powders was determined according to the AOAC official method 972.15 (AOAC, 2006). Moisture was determined according the procedure of AFNOR (NF VO4-282, December 1996).

Statistical Analysis

Data were analyzed by using the one-way analysis of variance (ANOVA), after testing normality and homogeneity of variance. Then, the "Tukey test" ($p<0.05$) was performed in order to find parameters that are significantly different from each other. Moreover, data being numerous, a principal component analysis (PCA) was made to find the main variation trends between fruits characters in the carob cultivars. In addition, hierarchical cluster analysis (HCA) was used to investigate the similarities and dissimilarities among the varieties. For classification, the Ward's Minimum Variance Method was utilized. The squared Euclidean distance was used as the dissimilarity measure for Ward's method. The freeware R (v. 2.13.1) was used for the statistical analysis. Furthermore, correlation between morphological, chemical parameters and environmental or

geographical factors (altitude, latitude, longitude) were evaluated using Pearson's correlation coefficient.

Results and discussion

Morphological characteristics

Results of morphological traits of carob pod are presented in Table 2. One-way ANOVA is performed on the data and significant differences between them were found.

Among the morphological characteristics, different notions can be distinguished: the pod size (which gathers pod length, width, thickness and size index), the pod weight (mass, volume, specific gravity) and the seed yield in a pod (number, weight).

The pod size

The pod length had been distinguished in four groups that varied from 11.42 ± 1.84 cm to 24.25 ± 1.84 cm. The longest were Khachabi, Akkari 1 and 2 (group 1), the shortest being Jnoubi 2 (group 4). For the pod width and thickness, the different groups, formed by the Tukey's test, were overlapped. However, Khachabi belongs to the group with the highest width and thickness while the wild type (Barri) was the smallest in width and thickness. This explained that Khachabi was the taller and pulpier and the wild type was less pulpy and as a result was the thinnest. This result was also found by other authors; wild types are known for their non-fleshy pulp with higher seed production and higher seed to husk ratio (Marakis et al., 1988; Ouchkif, 1988; Di Lorenzo, 1991; Battle and Tous, 1997; Gubbuk, 2010).

The pod weight

For the pod mass and volume, classification was the same; all the volume is full of matter. Barri and Jnoubi 2 got the smallest mass and volume whereas Khachabi was the heaviest and has the highest volume. However, the ratio between mass and volume leads to a different classification. Khachabi having a high mass and volume has a specific gravity inferior to Barri, which has a small mass and volume. Since Khachabi was the longest so for sure it occupies a larger space (volume) but it doesn't mean that it was the denser. Since density is the amount of matter crammed into a given space, obviously and statistically, Khachabi is presented as the less juicy pulp among the other Lebanese carob types. So pods as Barri and Jnoubi 2 are fully denser than big pods as Khachabi.

Seed yield in a pod

According to kernels, the seed number and weight give the same classification: Akkari 2 and Khachabi had the highest seed number; in the opposite, Jnoubi 2 has few seeds. The variable seeds yield /pod gives different classification than the two others where Barri has the highest value and Khachabi the lowest. This is explained by the fact that, the wild type Barri had small pulp and high seeds yield among all carob pod varieties.

Many authors mentioned that the carob wild type is highly rich in kernels than the cultivated type (Marakis et al., 1988; Di Lorenzo, 1991; Biner et al., 2007, Gubbuk et al, 2010).

Principal Component Analysis

Principal Component Analysis was performed to objectively interpret and compare the morphologic data of the carob samples and evaluate the most important variables able to discriminate carob. The application of PCA allowed reducing data to PC1 (50.83%), PC2 (20.13%) and PC3 (12.34%), which expressed 83% of the total variance of the data set. PC1 is obtained by combination of the length, the width, the pod weight, the volume, the seeds yield and the seeds weight/pod. These variables are coherent with the description of pod size; PC1 describes "big carob pods". This conclusion is coherent with the results of ANOVA, all these variables are linked and gives a same classification of carob. Then, PC2 is highly correlated with the seeds yield which describes the richness of seeds pod. Finally, PC3 is mainly explained by the specific gravity that described heavy pods with a low volume.

Furthermore, the individual factors map (Fig. 2) shows that it was difficult to separate varieties. We can see tendencies at the extremes: Khachabi correlated positively with PC1, thus it corresponds to big carob pods, Jnoubi2 is correlated negatively with PC1 and PC2, and it corresponds to small carob pods with less seeds. Barri is negatively correlated with PC1 and positively correlated with PC2. It corresponds to small carob pods with high seed content. Individuals are not well represented on the PC3, so this component was not interpreted.

Hierarchical cluster analysis

To investigate similarities between varieties and confirming PCA, analysis was completed by a hierarchical cluster analysis (Fig. 3). There are ten individuals by variety; thus, for almost all varieties, there is a gathering of the ten individuals except for Akkari 1 which is characterized by a high diversity inside this variety. Two clusters can be distinguished with two ungrouped varieties Barri and Khachabi, which confirms a high dissimilarity among all varieties particularly Barri the wild type and Khachabi.

Chemical characteristics

Table 3 presents the ten variables describing chemical characteristics of the carob pod. One-way ANOVA is performed on the data and significant differences between them are found. As in the literature, we found that carob pulp is highly rich in sugars (72.25 ± 0.50 for Khachabi to 89.46 ± 1.04 for Jnoubi 2), polyphenol (5.83 ± 0.91 for Khachabi to 21.87 ± 2.36 for Barri), in addition to an appreciable amount of fibre, and minerals (Custódio, 2011; Ayaz, 2007 Gruendel, 2007; Bengoechea, 2008; Zunft, 2001). The variable

protein content was low and not discriminative; it varied from 3.61 ± 0.72 (SM) to 5.62 ± 0.15 (Khachabi).

Principal Component Analysis

The application of PCA allowed reducing data to PC1 (31.92%), PC2 (29.42%) and PC3 (22.89%), which expressed 84% of the total variance of the data set. Thus, PC1 is obtained by combination of the sugar content and the fiber content. Then, PC2 is highly correlated with the polyphenol content and the mineral content. It describes carob with high nutritional properties. Finally, PC3 is explained positively by water content and negatively with protein content.

Furthermore, the individual factor maps (Fig. 4) allow separating some varieties. On figure 4a, we can see that the varieties Barri and Jnoubi are separated according the PC2. Wild type Barri had the highest polyphenol content whereas Khachabi had the lowest polyphenol content. On figure 4b, we see that Ahmar and SM are described by PC3, low water content and high protein content in opposition with Baladi.

Hierarchical cluster analysis

To investigate similarities between varieties, analysis was completed by a hierarchical cluster analysis (Fig. 5). Two groups and one ungrouped variety were discriminated. Wild carob was the ungrouped variety same as for morphological characteristics. The first group is composed of Jnoubi1, Jnoubi 2 and SM. The second group gathers the others varieties (Ahmar, Baladi, Khachabi, A1, A2).

Correlation between carob characteristics

The correlation matrix between morphological, chemical features and geographic parameters were summarized in Table 4. Pearson's coefficients were calculated and significant differences were found. The correlation analyses established by cultivar provided a specific understanding about the way how fruit, seed characteristics and geographical coordinates correlates within cultivar. Significant correlations were found between characteristics describing pod size, pod weight and seed yield. Thus, pods which are longer, heavier with a high volume and high size index have the highest seed number. Furthermore, seed yield was negatively correlated with pod width which explains that high seeds yield pods had the smallest width. These results agreed with those of Tous et al. (2009) and Barracosa et al. (2007), who showed that to achieve high seed yield, it is important to select thin, narrow carobs with pods that are not too heavy.

Besides, sugar content exhibited negative correlation with pod length, seeds number and weight which means that longest pod with heaviest and highest seed number furnished low sugar content. In addition, polyphenol content was positively correlated to seeds yield, so high polyphenol content is established by a high seeds yield.

Finally, the correlation between geographical coordinates and carob pod characters exhibited a highly positive and significant correlation between latitude and pod length weight volume, seeds weight and size index. It is coherent according to the repartition of varieties in Lebanon, country is more longer than larger. Thus, there is a significant difference on carob morphological characteristics according to the latitude. Big pods are located more in the north of the country and small pods in the south.

Conclusion

Carob seeds are the largest output of the locust bean gum in food industry. Thus, the industrial target is to get high seeds yield with high nutritional properties. Indeed, carob rich in sugars, polyphenols, fibre and minerals are interesting for health consumer particularly in food industry (dietary), medicinal and pharmacological industries (Custódio, 2011; Ayaz, 2007; Gruendel, 2007; Bengoechea, 2008; Zunft, 2001).

The relationship and correlations among all pods and seeds characters resulted in the separation of the 9 Lebanese populations into two grouped and two ungrouped populations. If we observed the two groups, we notice that there is a gathering according to latitude. The first group represents varieties with big pod size and high sugar content. These varieties are located in the north of Lebanon (A1, A2, Bal, Ah). The second group is less interesting in terms of nutritional properties. These varieties have small pod and are located in the south of Lebanon (J1, J2, SM). Furthermore, two varieties are very distinctive from the others and could have potential application for food industry: Khachabi has big pod size and high seed content with high sugar content and Barri the wild type which is characterized by small pods, high seed content, high sugar content and high polyphenol content. Finally, this study had brought supported information about Lebanese cultivars in order to help making breeding programs that helps for planting new carob orchards with best performing varieties.

Acknowledgments

The authors are grateful for the financial support of the Azm and Saadeh Foundation.

References:

AOAC. Official methods of Analysis of AOAC international. Gaithersburg. Maryland: AOAC 2006, 31: 931.04, 972.15.

AOAC. Official methods of Analysis of AOAC international. Gaithersburg. Maryland: AOAC 2007, 2: 955.04, 33: 990.19, 945.46.

Ayaz, F.A, Torun, H., Ayaz, S., Correia, P.J, Alaiz, M., Sanz, C., Gruz, J., Strnad, M., 2007. Determination of chemical composition of anatolian carob pod (Ceratonia Siliqua L.): sugars, amino and organic aids, minerals and phenolic compounds. J. food qual. 30 (6), 1040–1055.

Ayaz, F.A. & Torun, H., Glew, R.H., Bak, Z.D., Chuang, L.T., Presley, J.M. & Andrews, R., 2009. Nutrient Content of Carob Pod (Ceratonia siliqua L.) Flour Prepared Commercially and Domestically, Plant Foods Hum. Nutr. 64, 286–292.

Barracosa, P., Osorio, J., & Cravador, A., 2007. Evaluation of fruit and seed diversity and characterization of carob (Ceratonia siliqua L.) cultivars in Algarve region. Sci.Hort. 114, 250–257.

Batlle I., Tous J., 1997. Carob tree. Ceratonia siliqua L. Promoting the conservation and use of underutilized and neglected crops. 17, Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy. pp 92.

Bengoechea C., Romero A., Villanueva A., Moreno G., Alaiz M.,F. Millan , Guerrero A., Puppo M.C., 2008. Composition and structure of carob (Ceratonia siliqua L.) germ proteins, Food Chem. 107, 675–683.

Biner B., Gubbuk H., Karhan M., Aksu M., Pekmezci M., 2007. Sugar profiles of the pods of cultivated and wild types of carob bean (Ceratonia siliqua L.) in Turkey. Food Chem. 100, 1453–1455.

Caja, G., Albanell, E., Casanova, R., 1988. Caracterización morfológica de frutos de algarrobo cultivados en España. In: Proceedings of the II International Carob Symposium,Valencia, Spain, September 29–October 1, pp. 119–129.

Custódio, .L, Fernandes, E, Escapa, A.L, Fajardo, A., Aligue, R., Albericio, F., Neng. N.R., Nogueira, J.M., Romano, A. 2011. Antioxidant and cytotoxic activities of carob tree fruit pulps strongly influenced by gender and cultivar. J. Agric. Food Chem. 59, 7005–7012.

De Pádua, M., Fontoura, P.S.G. & Mathias, A.L., 2004. Chemical composition of Ulvaria oxysperma (Kützing) bliding, Ulva lactuca (Linnaeus) and Ulva fascita (Delile). Braz. arch. biol. technol. 47, 49–55.

Di Lorenzo, R., 1991. Carrubo. Frutticoltura speciale. Ed. REDA, Rome.

Dubois, M.K.A., Gilli, Y.K., Hamilton, P.A., (1956), Colometrie method for determination of sugari and related substances, Anal. chem.J. 28, 350– 356.

Girolamo, R., Laura, D., 2002. Evaluation and preservation of genetic resources of carob (Ceratonia siliqua L.) in southern of Italy for pharmaceutical use. Breeding Res. Aromatic Med.Plant. 9, 367–372.

Gruendel, S., Otto, B., Garcia, A.L., Wagner, K., Muelle, C., Weickert, M.O., Heldwein, W., Koebnick, C., 2007. Carob pulp preparation rich in insoluble dietary fibre and polyphenols increases plasma glucose and serum insulin responses in combination with a glucose load in humans Br. J. Nutr. 98 (1), 101–105.

Food and Agriculture Organization of the United Nations (FAO). (2001). Non-wood Forest Products in the Near East: A Regional and National Overview. FAO Corporate Document Repository, 3.Country reports, 3.8,

Lebanon. Retrieved on 12-18-2006 from http://www.fao.org/docrep/003/Y1797E/y1797e13.htm
Gubbuk, H., Kafkas, E., Guven, D., Gunes, E., 2010. Physical and phytochemical profile of wild and domesticated carob. Span. J. Agric. Res. 8(4), 1129–1136.
Hariri, A., Ouis, N., Sahnouni, F., Bouhadi, D., 2009. Mise en oeuvre de la fermentation de certains ferments lactiques dans des milieux a base des extraits de caroube. Rev. microbiol. ind. san et environn., 37-55.
Lossaint, P. (1973). Soil-vegetation relationships in Mediterranean ecosystems of southern France. In: Castri, F., Mooney, H.A. (Eds.), Mediterranean type ecosystems origin and structure. Heidelberg Press, New York, pp. 199–210.
Marakis, S., Kalaitzakis J.& Mitrakos K. 1988. Criteria for recognizing carob tree varieties. In: Fito, P., Mulet, A. (Eds.), Proceedings of the II International Carob Symposium Valencia, Spain, pp. 558-566.
Markis, D.P.& Kefalas, P., 2004. Carob pods (Ceratonia siliqua L.) as a source of polyphenolic antioxydants. Food Technol. Biotechnol. 42 (2), 105–108.
Neukom, H. 1989. Galactomannans: Properties and applications. Lebensm. Wiss. Technol. 22, 41–45.
Naghmouchi S., Khouja M.L., Romero A., Tous J., Boussaid M. 2009. Tunisian carob (Ceratonia siliqua L.) populations: Morphological variability of pods and kernel. Sci.Hort.121, 125–130.
NAS, 1979. Tropical Legumes: Resources for the Future. National Academy of Sciences, Washington DC, USA, pp. 109–116.
Owen R.W., Haubner R., Hull W.E., Erben G., Spiegelhalder B., Bartsch H., Haber B., 2003. Isolation and structure elucidation of the major individual polyphenols in carob fibre. Food Chem. Toxicol. 41, 1727–1738.
Petit, M.D., Pinilla, J.M., 1995. Production and purification of a sugar syrup from carob pods, LWT-Food Sci. Technol. 28, 145–152.
Sandolo, C., Coviello, T., Matricardi, P., Alhaique, F., 2007. Characterization of polysaccharide hydrogels for modified drug delivery. Eur. Biophys. J. 36 (7), 693–700.
Shawakfeh, K. and Ereifej, K. I., 2005. Pod Characteristics of two Ceratonia siliqua L. Varieties from Jordan. Ital. J. Food Sci. 17 (2):187–194.
Sidina M.M., El Hansali M., Wahid N., Ouatmane A., Boulli A., Haddioui A., 2009. Fruit and seed diversity of domesticated carob (Ceratonia siliqua L.) in Morocco. Sci.Hort. 123, 110–116.
Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am. J. Enol. Viticul., 16, 144–158.

Thomson, P., 1971. The carob in California. California Rare Fruit Growers Yearbook 3, 61–102.
Winer, N., 1980. The potential of the carob (Ceratonia siliqua L.). Int.tree crop. J. 1, 15–26.
Zunft, H.J, Luder, W., Harde A., Haber, B., Graubaum, H.J., Gruenwald, J., 2001. Carob pulp preparation for treatment of hypercholesterolemia. Adv. Ther. 18, 1230–236.
Tous, J., Romero, A., Hermoso, J.F., Ninot, A., Plana, J., Batlle, I., 2009. Agronomic and commercial performance of four Spanish carob cultivars. HortTechnol. 19, 465–470.

Figure 1. Repartition map of selected Lebanese carob populations. Abbreviations indicate populations: (A1) Akkari 1; (A2) Akkari 2; (Ah) Ahmar; (Bal) Baladi; (Bar) Barri, (J1) Jnoubi1; (J2) Jnoubi2; (Kh) Khachabi; (SM) Sandali Makdissi.

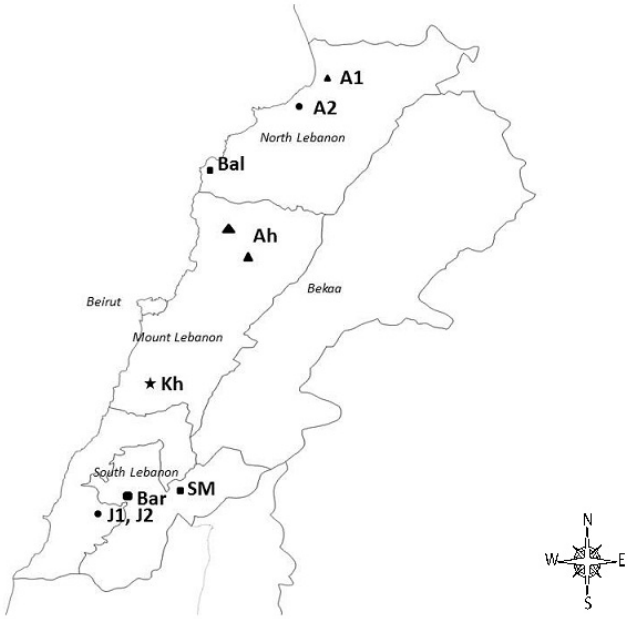

Figure 2. Pod size and seeds separation according to individual factor map (PC1 and PC2).

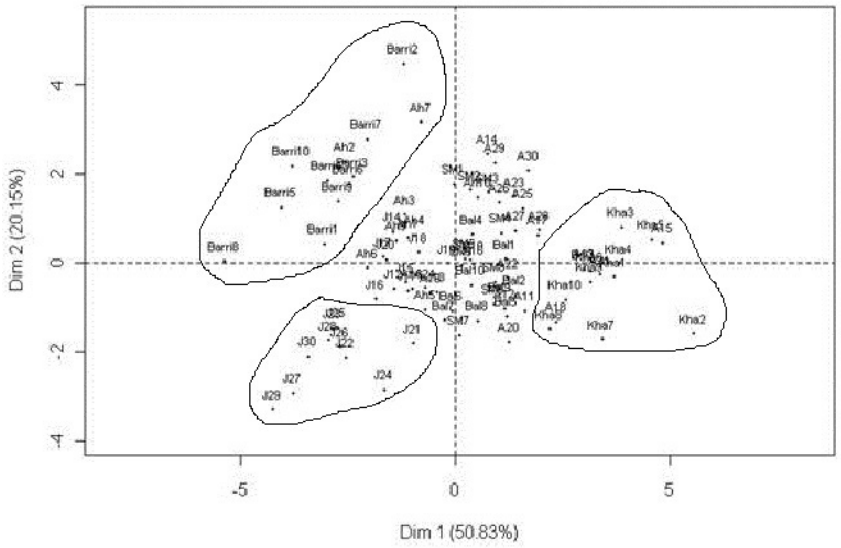

Figure 3. Hierarchical Ascendant Classification on morphological characters. Group 1 : A1, A2 and Bal ; Group 2 : J1, J2, AH and SM - Khachabi and Barri are ungrouped.

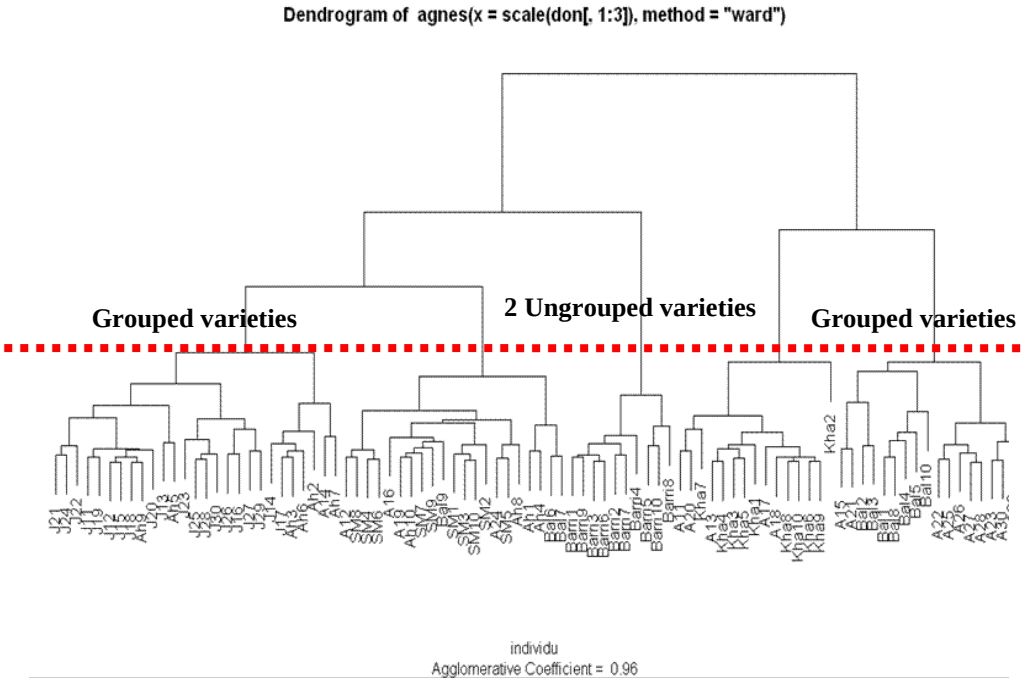

Figure 4.a. polyphenol content separation according to individual factor map (PC1 and PC2).

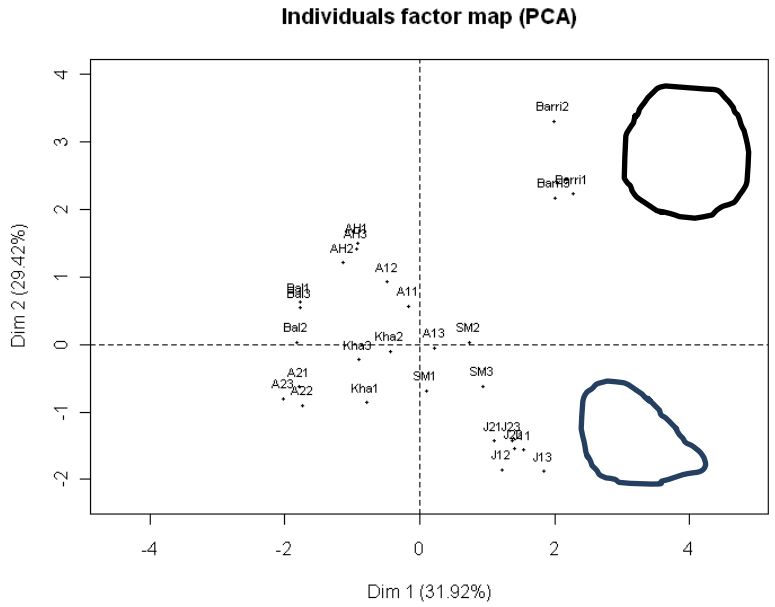

Figure 4.b

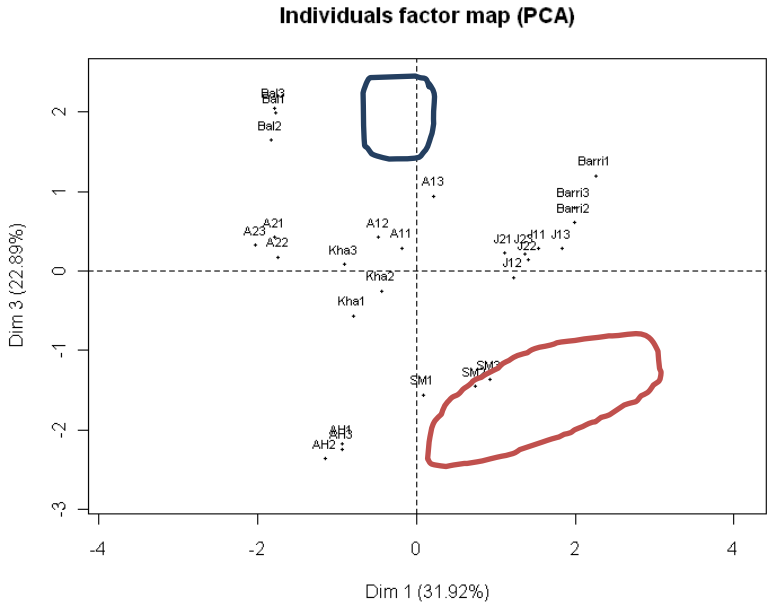

Figure 5. Hierarchical Ascendant Classification on chemical characters. Group 1: A1, A2, Bal, AH and Kh; Group 2 : J1, J2 and SM; ungrouped variety Barri.

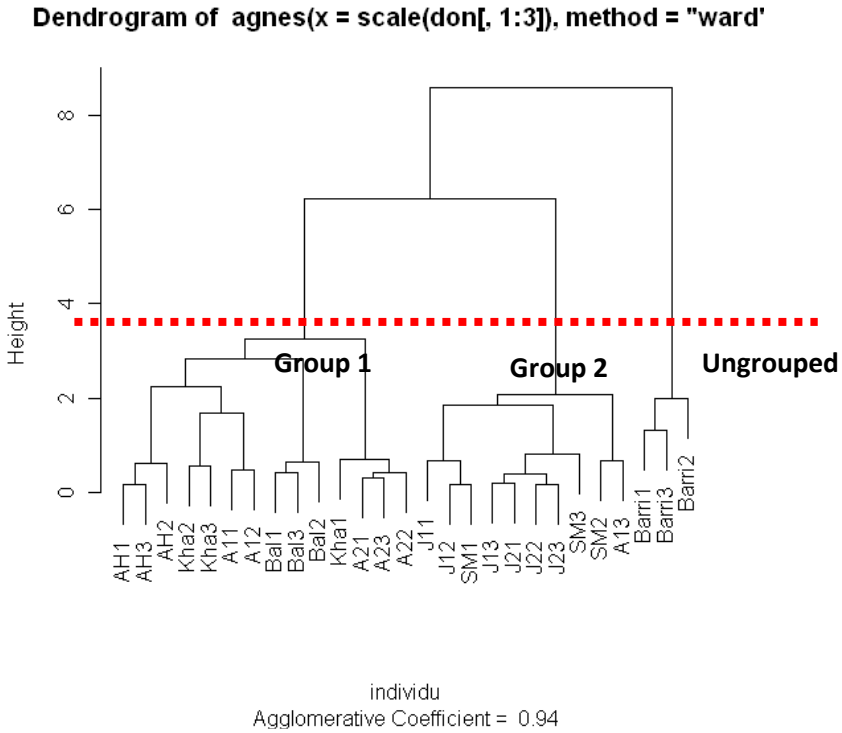

Table 1. GPS Locations of the analyzed Lebanese carob varieties.

Variety	Elevation (feet)	Elevation (m)	Latitude N		Longitude E		Regions
Akkari 11	600	182.88	34°	30.841'	36°	01.948'	Akkar
Akkari 22	786	239.57	34°	27.641'	35°	57.238'	Akkar
Ahmar	188	57.30	34°	17.259'	35°	40.256'	Batroune
Baladi	663	202.082	34°	16.693'	35°	40.315'	Selaata
Khachabi	1350	411.48	33°	39.065'	35°	28.789'	Bourjen
SM	2147	654.41	33°	22.906'	35°	35.943'	Marjayoun
Jnoubi 1	356	108.509	33°	18.532'	35°	16.865'	Maaroub and Borj Rahal
Jnoubi 2	1574	479.76	33°	15.014'	35°	25.247'	Maaroub and Borj Rahal
Barri	1760	536.45	33°	15.424'	35°	27.710'	Wadi El Hojeir

° = Degree, '= minutes

Table 2. Mean values of the Physical measurements of carob pods populations

	A1	A2	Ah	Bal	Bar	J1	J2	SM	Kh
Pod length (cm)	21.88 ± 2.44^{a}	22.82 ± 2.42^{a}	16.06 ± 1.61^{c}	19.14 ± 1.41^{b}	15.71 ± 1.45^{c}	14.66 ± 0.86^{c}	11.42 ± 1.84^{d}	18.91 ± 1.78^{b}	24.25 ± 1.84^{a}
Pod width(cm)	2.34 ± 0.18^{bc}	2.50 ± 0.16^{ab}	2.04 ± 0.25^{d}	2.74 ± 0.27^{a}	1.73 ± 0.11^{e}	2.02 ± 0.12^{d}	2.14 ± 0.15^{cd}	2.23 ± 0.12^{bcd}	2.49 ± 0.16^{ab}
Pod thickness (cm)	0.85 ± 0.08^{ab}	0.65 ± 0.06^{de}	0.71 ± 0.10^{cde}	0.60 ± 0.11^{e}	0.48 ± 0.04^{f}	0.80 ± 0.07^{bc}	0.76 ± 0.06^{bcd}	0.76 ± 0.04^{bcd}	0.92 ± 0.11^{a}
Pod weight (g)	27.70 ± 7.05^{b}	26.71 ± 5.80^{bc}	16.45 ± 3.76^{de}	27.14 ± 4.57^{b}	8.93 ± 1.67^{f}	17.72 ± 2.08^{de}	13.38 ± 3.62^{e}	20.91 ± 3.02^{cd}	36.85 ± 3.97^{a}
Pod volume(cm^3)	33.50 ± 9.52^{b}	26.60 ± 4.62^{c}	16.80 ± 2.82^{d}	29.1 ± 2.92^{bc}	9.95 ± 1.91^{e}	18.20 ± 3.08^{d}	14.22 ± 3.43^{de}	27.70 ± 3.33^{bc}	47.90 ± 4.68^{a}
Specific gravity	0.83 ± 0.05^{cd}	1 ± 0.06^{a}	0.97 ± 0.10^{ab}	0.93 ± 0.10^{abc}	0.90 ± 0.03^{bc}	0.98 ± 0.10^{ab}	0.94 ± 0.08^{abc}	0.76 ± 0.11^{d}	0.77 ± 0.01^{d}
Size index	9.43 ± 1.26^{a}	9.15 ± 0.93^{ab}	7.95 ± 1.22^{bcd}	7.03 ± 0.62^{d}	9.3 ± 0.94^{ab}	7.28 ± 0.60^{cd}	5.39 ± 0.53^{e}	8.51 ± 0.95^{abc}	9.75 ± 0.84^{a}
No.seeds/pod	12.40 ± 2.5^{ab}	13.90 ± 2.28^{a}	12.90 ± 2.47^{ab}	13.1 ± 1.97^{ab}	10.60 ± 2.95^{bc}	12.10 ± 0.99^{ab}	7.4 ± 1.78^{c}	13.10 ± 1.97^{ab}	14.10 ± 2.08^{a}
Seeds weight	2.73 ± 0.65^{ab}	3.02 ± 0.47^{a}	2.59 ± 0.63^{ab}	2.72 ± 0.81^{ab}	1.80 ± 0.55^{cd}	2.39 ± 0.22^{bc}	1.36 ± 0.30^{d}	2.72 ± 0.81^{ab}	3.10 ± 0.43^{ab}
% seeds/pod	9.85 ± 2.82^{cd}	11.30 ± 4.72^{bcd}	15.76 ± 4.58^{ab}	10.02 ± 3.03^{cd}	20.10 ± 3.44^{a}	13.47 ± 1.24^{bc}	10.14 ± 1.84^{cd}	13.00 ± 4.81^{bc}	8.41 ± 1.16^{d}

Values are the average of 10 pods, standard deviations are indicated below. Letters ^{abcde} show the significant differences ($p<0.05$)

Table 3. Mean values of the Physico-chemical measurements of carob pods populations

	A1	A2	Ah	Bal	Bar	J1	J2	SM	Kh
Eau	14.37±0.05^{c}	13.89±0.05^{d}	17.05±0.05^{a}	13.86±0.05^{d}	14.40±0.05^{c}	13.54±0.05^{e}	13.58±0.05^{e}	15.47±0.05^{b}	14.37±0.05^{c}
Sucres	78.38±6.54^{bc}	74.50±0.75^{c}	72.33±0.54^{c}	81.17±0.87^{abc}	81.98±6.29^{abc}	85.33±3.02^{ab}	89.46±1.04^{a}	85.01±3.83^{ab}	72.2±0.50^{c}
Protéines	4.80±0.16^{b}	4.81±0.14^{b}	4.08±0.03^{d}	5.62±0.15^{a}	4.26±0.13^{cd}	4.18±0.07^{d}	3.92±0.08^{de}	3.61±0.72^{e}	4.62±0.23^{bc}
Fibres	6.11±0.01^{d}	5.28±0.03^{g}	5.70±0.01^{f}	4.80±0.02^{h}	6.60±0.02^{b}	7.74±0.01^{a}	6.61±0.02^{b}	5.87±0.03^{e}	6.41±0.02^{c}
Cendres	2.99±0.01^{b}	2.46±0.06^{d}	2.65±0.01^{cd}	2.68±0.08^{bcd}	3.56±0.16^{a}	2.64±0.09^{cd}	2.65±0.01^{cd}	2.82±0.21^{bc}	2.77±0.17^{bcd}
Polyphénols	10.90±1.43^{bc}	6.22±0.58^{d}	13.26±1.50^{b}	12.92±1.22^{b}	21.87±2.36^{a}	7.09±0.52^{d}	6.61±0.38^{cd}	6.73±0.10^{d}	5.83±0.91^{d}

Values are the average of 3 pods, standard deviations are indicated below. Letters ^{abcde} show the significant differences ($p<0.05$)

Table 4. Pearson's correlations between morphological, chemical and geographical parameters

	L	**W**	**Th**	**Mp**	**Vol**	**SG**	**Si**	**Sn**	**Ms/p**	**Ps/p**	**S**	**Ppol**	**P**	**F**	**A**	**H2O**	**H**	**Lat**	**Long**
L	1.000	0.660	0.215	0.876**	0.856**	-0.388	0.738*	0.813**	0.875**	-0.450	-0.680*	-0.260	0.510	-0.442	-0.106	0.011	-0.072	0.866**	0.172
W		1.000	0.183	0.827**	0.737*	-0.149	-0.002	0.517	0.630	-0.809**	-0.301	-0.476	0.705*	-0.702*	-0.529	-0.207	-0150	0.481	0.323
Th			1.000	0.508	0.601	-0.472	0.114	0.161	0.282	-0.632	-0.125	-0.643	-0.203	0.379	-0.337	0.028	-0.086	0.588	-0.533
Mp				1.000	0.970**	-0.363	0.444	0.727*	0.834**	-0.778	-0.579	-0.529	0.562	-0.355	-0.400	-0.131	-0.189	0.852**	0.064
Vol					1.000	-0.571	0.481	0.669*	0.776*	-0.738	-0.505	-0.512	0.420	-0.257	-0.261	-0.076	-0.014	0.895**	-0.048
SG						1.000	-0.324	-0.131	-0.185	0.230	-0.090	0.152	0.201	-0.037	-0.378	-0.114	-0.664	-0.532	0.334
Si							1.000	0.681*	0.648	0.125	-0.645	0.094	0.122	0.066	0.309	0.147	-0.052	0.687*	-0.085
Sn								1.000	0.978*	-0.238	-	-	0.391	-	-	0.29	-	0.594	0.333

									*		0.789*	0.236		0.409	0.299	0	0.335		
Ms/p									1.000	-0.416	-0.769*	-0.368	0.426	-0.431	-0.382	0.216	-0.332	0.703*	0.276
Ps/p										1.000	0.092	0.766*	-0.401	0.228	0.633	0.371	0.133	-0.571	0.080
S											1.000	0.015	-0.363	0.295	0.219	-0.420	0.534	-0.573	-0.295
Ppol												1.000	0.134	-0.035	0.784	0.213	-0.034	-0.470	-0.123
P													1.000	-0.543	-0.120	-0.372	-0.452	0.220	0.129
F														1.000	0.239	-0.261	0.081	-0.051	-0.629
A															1.000	0.034	0.441	-0.133	-0.403
H2O																1.000	-0.107	-0.059	0.211
H																	1.000	0.020	0.044
Lat																		1.000	-0.117
Long																			1.000

* significant differences at p<0.05,** significant differences at p<0.01; L:Length, W: weight, Th: Thickness, Mp:mass of the pod, Vol: volume: SG: Specific Gravity, Si:Size index, Sn: Seed number, Ms/p: Masse seed /pod, Ps/p: percentage of seeds /pod, S: Sugar, Ppol: Polyphenol, F: Fiber, A: Ash, H2O: Humidity, H:Hight, Lat: Latitude, Long: Longitude.